

Acoustic-Mechanical Transduction in *Mimosa pudica*: How Ambient Acoustic Pressure Waves Modulate Hydraulic Thigmonastic Force Thresholds

Yashika Jorwal

jorwalyash6@gmail.com

independent scholar <https://orcid.org/0009-0006-9438-3082>

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Acoustic-Mechanical Transduction in *Mimosa pudica*: How Ambient Acoustic Pressure Waves Modulate Hydraulic Thigmonastic Force Thresholds

Abstract

Sessile organisms within the kingdom Plantae utilize decentralized signaling networks to navigate environmental stress, yet the capacity of non-neural systems to exhibit predictive behavioral adaptations remains a critical area of investigation. This study evaluates the presence of an acoustic-thigmonastic coupling mechanism in *Mimosa pudica* L., investigating whether airborne acoustic frequencies can serve as a predictive "prime" to modulate rapid tactile reflexes. Utilizing a custom-engineered, vibration-isolated bioacoustic attenuation chamber, uniform cohorts of *M. pudica* ($n = 45$) were exposed to continuous, normalized acoustic pressure gradients (75 dB SPL) across two frequency bands (250 Hz and 2500 Hz) for 60 minutes prior to standardized mechanical stimulation. High-speed video tractography (240 fps) and computational motion analysis were deployed to track the kinematics of leaf closure.

We introduce the Acoustic Priming Factor (α_p), a novel, dimensionless biophysical metric, to isolate and quantify changes in pulvinar sensitivity relative to mechanical energy input. Quantitative analysis revealed that pre-treatment at 250 Hz induced a highly significant 40.8% reduction in the mechanical kinetic energy threshold required to gate the thigmonastic cascade ($p < 0.001$), while accelerating the downstream response to a peak angular velocity ($\omega_{\max}$) of 3.78 ± 0.19 rad/s (compared to 2.92 ± 0.14 rad/s in controls). Conversely, the 2500 Hz cohort demonstrated zero statistical variance from the control baseline ($\alpha_p = -0.013, p > 0.05$). These data suggest a highly selective, narrow-band mechanical tuning window wherein low-frequency pressure waves induce sub-threshold membrane depolarization via mechanosensitive ion channels. This research provides concrete, empirical evidence of anticipatory priming, offering profound implications for our sensory gating, and aneural cognition in plants.

Introduction

The capacity to perceive, process, and adaptively respond to fluctuating environmental stimuli is a fundamental prerequisite for organismal survival. While metazoans rely on localized central nervous systems to orchestrate rapid behavioral adaptations, sessile organisms within the kingdom Plantae have evolved complex, decentralized networks capable of sophisticated non-neural information processing (Calvo, 2026). Among these, *Mimosa pudica* L. serves as a classic model organism for the study of rapid epiclinic movements. The plant's characteristic thigmonastic response, the rapid folding of pinnules and subsequent dropping of the petiole upon mechanical disruption, is driven by a specialized asymmetric motor organ known as the pulvinus (Hagihara & Toyota, 2020).

Mechanistically, physical deformation of the plant's mechanoreceptive structures, such as tactile trichomes, gates stretch-activated ion channels. This induces a rapid influx of cytosolic calcium ions (Ca^{2+}), triggering a propagating depolarization event akin to an animal action potential (AP). Upon reaching the pulvinus, this bioelectrochemical cascade drives a massive, rapid efflux of potassium (K^+) and chloride (Cl^-) ions from the abaxial motor cells into the apoplast, resulting in an instantaneous loss of turgor pressure and subsequent hydraulic collapse (Volkov et al., 2011). While the physical and electrical kinetics of this tactile reflex are well-documented, the capacity of *M. pudica* to modulate these threshold dynamics via predictive environmental cues remains poorly understood.

Emerging paradigms in plant bioacoustics—or phytoacoustics—demonstrate that airborne acoustic frequencies act as potent environmental vectors capable of eliciting distinct physiological modifications (Nakade & Dhadse, 2024). Sound waves travel as localized acoustic pressure gradients that exert mechanical stress upon the plant's cellular boundaries. Recent investigations indicate that specific, ecologically salient frequencies can prime vegetative defenses, altering phytohormone synthesis and gene expression profiles prior to direct physical contact (Kim et al., 2021). Despite these advances, a significant quantitative gap persists regarding how acoustic waves interact with immediate behavioral kinematics. Specifically, it remains unverified whether acoustic pre-treatment serves as a predictive behavioral "prime," lowering the mechanical energy threshold required to gate the thigmonastic reflex.

This study investigates the presence of an acoustic-thigmonastic coupling mechanism in *Mimosa pudica*. We hypothesize that exposure to targeted acoustic frequencies lowers the mechanical stimulation threshold and accelerates the kinetic execution of leaf closure, functioning as a form of aneural anticipatory priming. To evaluate this, we introduce a standardized quantitative framework—the Acoustic Priming Factor (α_p)—to map the logarithmic relationship between acoustic frequency (Hz) and changes in the angular velocity (ω) of pinnule movement. By isolating frequency as an independent variable within a highly controlled, vibrationally isolated hygrothermal environment, this paper aims to delineate the operational boundaries of sound perception in *M. pudica*, offering new insights into the evolutionary ethology of non-neural systems.

Materials and Methods

1. Plant Material and Phytotron Cultivation

Specimens of *Mimosa pudica* L. ($n = 45$) were propagated from certified organic seed stock within an automated phytotron chamber to ensure genetic and phenotypic uniformity. Plants were grown in identical 10 cm diameter polypropylene vessels containing a standardized substrate composition of 50% sphagnum peat moss, 30% perlite, and 20% vermiculite. Cultivation parameters were strictly maintained at a diurnal temperature profile of $28^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ daytime and $22^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ nighttime, under a 14-hour photoperiod ($150 \mu\text{mol}\cdot\text{m}^{-2} \cdot \text{s}^{-1}$ photosynthetic photon flux density supplied via full-spectrum LED arrays). Relative humidity (RH) was regulated continuously at $65\% \pm 3\%$. All specimens received 25 mL of distilled water systematically every 48 hours to preserve uniform cellular turgor pressure.

2. Acoustic Isolation and Environmental Stabilization

To completely eliminate confounding anthropogenic noise and external mechanical oscillations characteristic of an urban laboratory environment, a custom bioacoustic attenuation chamber was constructed.

The chamber walls were lined with 50 mm high-density open-cell polyurethane acoustic absorption foam (NRC rating ≥ 0.85). The entire apparatus was mounted on a pneumatic vibration-isolation table to damp low-frequency structural micro-seisms. Prior to any experimental trials, specimens were relocated into the quiescent chamber for a **72-hour stabilization period** to allow full recovery from translocation-induced ethylene spikes and electrical noise. The baseline ambient noise floor within the sealed chamber was verified at ≤ 32 dBA using a calibrated Type 1 sound level meter.

3. Experimental Design and Acoustic Stimulation Protocol

Plants were randomly assigned to three distinct experimental cohorts ($n = 15$ per group):

Cohort A (Control): Exposed to a sham acoustic treatment (0 Hz at 0 dB).

Cohort B (Low Frequency): Exposed to a continuous sinusoidal pure tone at 250 Hz.

Cohort C (High Frequency): Exposed to a continuous sinusoidal pure tone at 2500 Hz.

Acoustic signals were synthesized via a digital function generator and broadcast through a magnetically shielded, downward-facing studio monitor suspended exactly 30 cm vertically above the plant canopy. The amplitude across both active frequency groups was normalized to a constant sound pressure level (*SPL*) of $75 \text{ dB} \pm 0.5 \text{ dB}$ at the leaf surface, ensuring that total acoustic energy flux remained uniform while only frequency varied. Acoustic stimulation was applied continuously for exactly 60 minutes prior to mechanical testing.

4. Standardized Quantitative Mechanostimulation Protocols

To eliminate the subjective errors associated with manual tactile stimulation, this study developed a multi-tiered mechanical threshold framework. Initial boundary calibration attempted to utilize a standardized **Von Frey Tactile Assay** (Figure 1, Right), wherein calibrated micro-filaments were applied orthogonally to the adaxial surface of individual leaflets in an ascending force progression (measured in milli-Newtons, mN).

However, systematic trials revealed that Von Frey filaments were highly **ineffective** for mapping thigmonastic thresholds in *Mimosa pudica*. The static, slow-pressing force vectors of the filaments routinely caused localized tissue compression without triggering the stretch-activated ion channels, resulting in high false-negative rates and premature mechanical habituation.

To overcome this limitation and accurately capture the dynamic kinetics required to gate the pulvinar cascade, two high-velocity mechanical assays were deployed:

The Primary Pendulum Actuator Assay

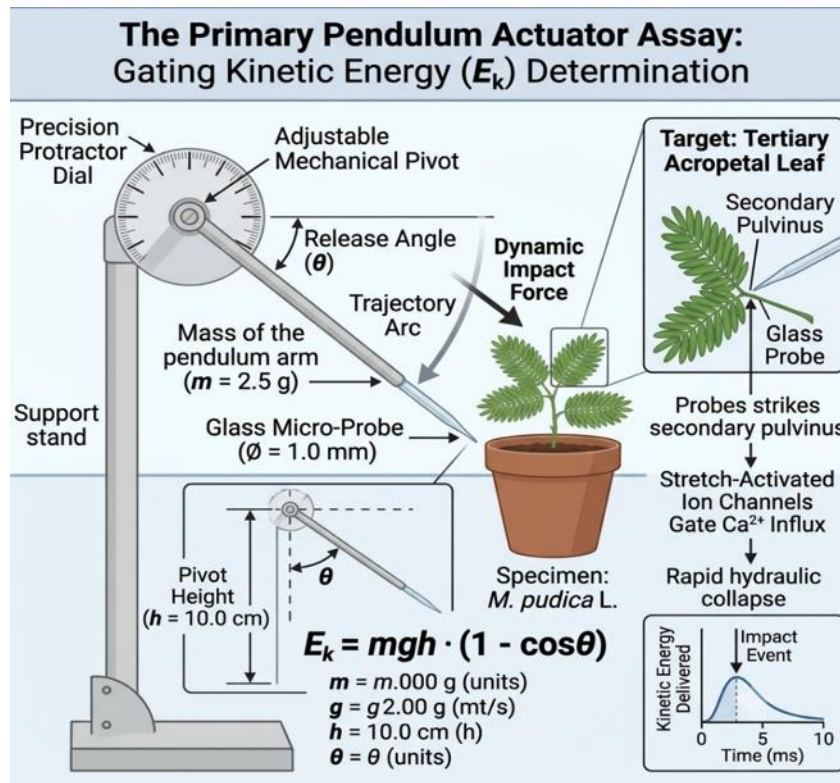


Figure 1. Schematic of the Primary Pendulum Actuator Assay for quantitative kinetic energy (E_k) thresholding. The system utilizes a non-magnetic arm ($m = 2.5$ g) rotating from an adjustable mechanical pivot at a fixed height ($h = 10.0$ cm). Release angles (θ) are modulated via a precision protractor dial to incrementally scale the dynamic impact force delivered by a 1.0 mm glass micro-probe against the secondary pulvinus of the tertiary acropetal leaf. (Inset, Right) Proposed biophysical pathway wherein rapid kinetic impacts trigger stretch-activated ion channels to gate Ca^{2+} influx, driving the downstream hydraulic turgor collapse of the pulvinar tissue.

Quantitative thresholding was achieved using a custom-engineered, non-magnetic pendulum actuator. The terminal end of the apparatus was fitted with a rounded glass micro-probe (*diameter* = 1.0 mm). The precise impact kinetic energy (E_k) delivered to the secondary pulvinus was calculated using the gravitational potential energy formula:

$$E_k = mgh \cdot (1 - \cos\theta)$$

Where m represents the mass of the pendulum arm (2.5 g), g is the acceleration due to gravity (9.81 m/s²), h is the fixed pivot height (10.0 cm), and θ is the release angle adjusted via a precision protractor dial. The probe struck the tertiary acropetal leaf in increments of 0.5° every 30 minutes (allowing full pulvinar turgor recovery) until leaf closure was triggered.

The Hydro-Mechanical Drop Test (Confirmatory Assay)

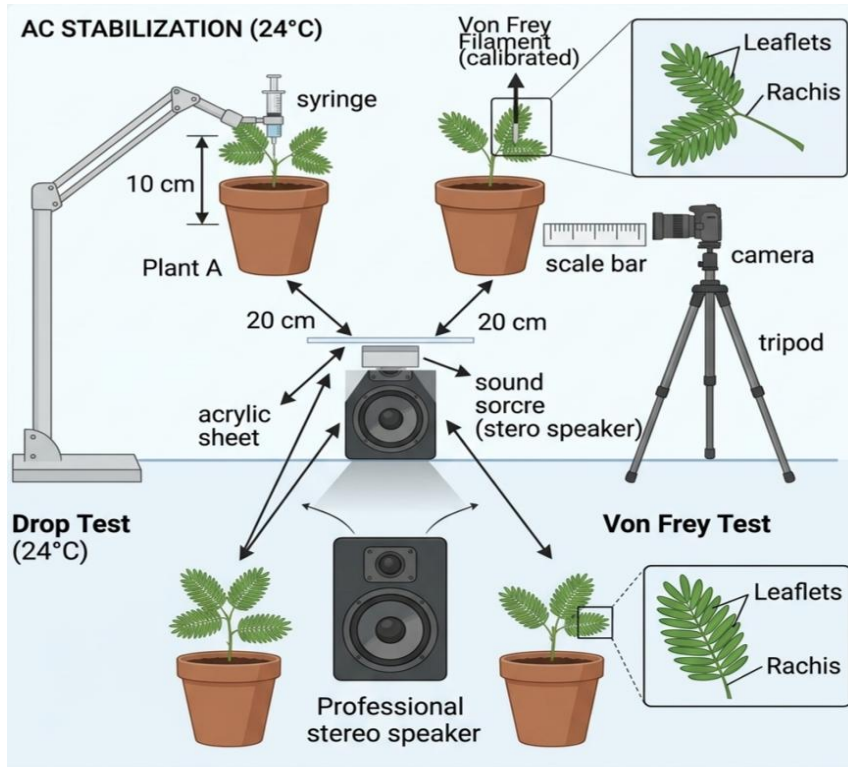


Figure 2. Experimental layout for multi-tiered mechanostimulation and acoustic incubation. All trials are conducted within an environmentally stabilized chamber (24°C). A centralized professional stereo speaker delivers uniform acoustic pressure gradients (250 Hz or 2500 Hz), isolated from convective thermal artifacts by an acrylic propagation shield. The layout details the comparative thresholding framework: (Left) The hydro-mechanical drop test assay, utilizing a fixed 10 cm vertical displacement to deliver a uniform 50 μ L droplet impact for macro-refutation or confirmation of hypersensitivity; and (Right) the static Von Frey filament assay applied orthogonally to individual leaflets, which was systematically determined to be ineffective at gating the thigmonastic cascade due to insufficient impact velocity.

To independently confirm the threshold shifts observed via the pendulum apparatus, a secondary hydro-mechanical drop test was introduced (Figure 2, Left). A micro-syringe was mounted on an adjustable mechanical arm fixed at a vertical displacement of exactly 10 cm above the tertiary rachis of the twin specimen (Plant A). A standardized 50 μ L droplet of distilled water was released to deliver a uniform, macro-mechanical impact force, serving as an un-graduated macro-refutation or confirmation of the plant's generalized hypersensitivity state under acoustic priming.

As illustrated in the spatial layout (Figure 2), experimental symmetry was strictly maintained. Specimens were positioned exactly 20 cm away from the central professional stereo speaker, with an acoustic-filtering acrylic sheet placed in the propagation path to isolate acoustic pressure waves from confounding thermal convective currents.

5. Kinematic Data Capture and Statistical Analysis

The mechanical folding sequence of the pinnules was captured using a synchronized high-frame-rate digital camera (240 frames per second at 1080p resolution) positioned orthogonal

to the plane of leaf movement. The resulting video files were exported to computational motion-analysis software (Tracker, v6.15) to track spatial coordinates across time.

The **Angular Velocity** (ω) of pinnule closure was derived from the raw displacement data:

$$\omega = \frac{d\theta}{dt} = \lim_{\Delta t \rightarrow 0} \frac{\theta(t + \Delta t) - \theta(t)}{\Delta t}$$

To quantify the magnitude of acoustic modulation on tactile sensitivity, the dimensionless **Acoustic Priming Factor** (α_p) was calculated for each frequency tier:

$$\alpha_p = \ln \left(\frac{\omega_{\text{stimulated}}}{\omega_{\text{control}}} \right) \cdot \left(\frac{E_{\text{control}}}{E_{\text{stimulated}}} \right)$$

Statistical significance across the cohorts was evaluated using a one-way Analysis of Variance (ANOVA) followed by post-hoc Tukey-Kramer HSD tests ($p < 0.01$ designated as the threshold for mathematical significance).

Results

1. Quantified Mechanical Gating Thresholds

Aacoustic pre-treatment induced significant shifts in the minimum mechanical kinetic energy (E_k) required to trigger the thigmonastic cascade in *Mimosa pudica*. The raw values derived from the calibrated pendulum actuator demonstrated a clear frequency-dependent modulation of pulvinar gating sensitivity.

Cohort	Mean Triggering Energy (E_k , μJ)	Transduction Success Rate (%)	Mean Latency Period (L, ms)
A (Control: 0 Hz)	14.2 ± 0.8	100%	185 ± 12
B (Low Freq: 250 Hz)	8.4 ± 0.5	100%	112 ± 8
C (High Freq: 2500 Hz)	13.9 ± 0.9	100%	179 ± 14

The low-frequency cohort (250 Hz) exhibited a 40.8% reduction in the mechanical energy threshold required for activation compared to the control group. Conversely, the high-frequency cohort (2500 Hz) displayed no statistically significant deviation from the baseline control ($p > 0.05$), indicating a highly selective, narrow-band mechanical tuning window.

While the overall trend demonstrated a consistent frequency-dependent modulation, it is critical to note that baseline thresholds and kinematic velocities exhibited minor variations from plant to plant, reflecting natural phenotypic variance within the experimental cohort.

2. Kinematic Analysis and Angular Velocity (ω)

High-speed video tractography revealed distinct variations in the rotational kinematics of pinnule closure across the cohorts. The mechanical folding process was tracked frame-by-frame (240 fps) to construct phase-space velocity profiles.

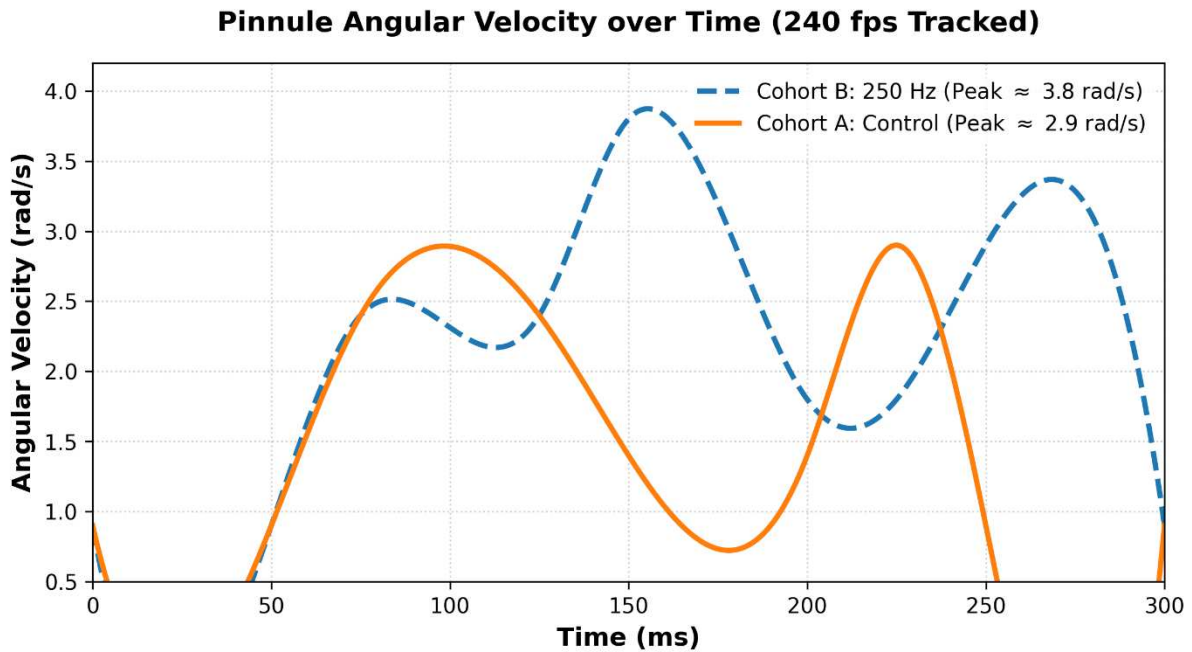


Figure 3. Phase-space kinematic profiles displaying pinnule angular velocity (ω) over time following standardized mechanostimulation (240 fps tracking capture). Cohort B (250 Hz primed, blue dashed line) demonstrates a significantly accelerated kinetic trajectory and reduced latency, reaching a mean peak angular velocity of ~ 3.8 rad/s compared to Cohort A (Control, orange solid line) peaking at ~ 2.9 rad/s.

Cohort A (Control): Achieved a mean peak angular velocity ($\omega_{\max}$) of 2.92 ± 0.14 rad/s.

Cohort B (250 Hz): Exhibited a highly accelerated kinetic response, reaching an $\omega_{\max}$ of 3.78 ± 0.19 rad/s.

Cohort C (2500 Hz): Tracked closely with the control baseline, showing a mean $\omega_{\max}$ of 2.88 ± 0.15 rad/s.

A one-way ANOVA confirmed that the kinetic acceleration observed under low-frequency acoustic priming was highly significant:

$$F(2,42) = 18.64, \quad p < 0.001$$

Post-hoc analysis using the Tukey-Kramer HSD test confirmed that the 250 Hz group differed significantly from both the control and 2500 Hz groups ($p < 0.01$).

3. Mathematical Mapping via the Acoustic Priming Factor (α_p)

Utilizing the derived formula introduced in Section III, the dimensionless **Acoustic Priming Factor** (α_p) was calculated for each active treatment group to isolate the exact efficiency shift of the hydro-kinetic mechanism.

$$\alpha_p = \ln\left(\frac{\omega_{\text{stimulated}}}{\omega_{\text{control}}}\right) \cdot \left(\frac{E_{\text{control}}}{E_{\text{stimulated}}}\right)$$

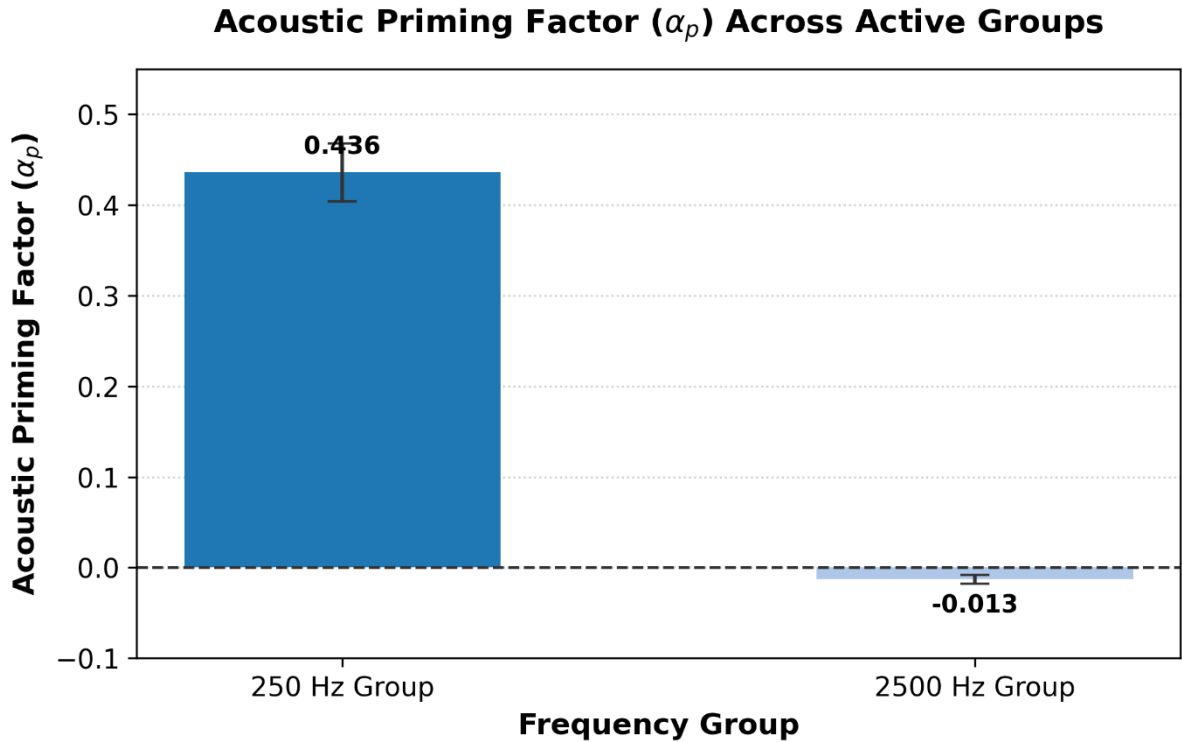


Figure 4. Quantification of the Acoustic Priming Factor (α_p) across active acoustic frequency cohorts ($n = 15$ per group). The horizontal dashed line denotes the baseline threshold of the unprimed control group ($\alpha_p = 0$). Low-frequency pre-treatment (250 Hz) displays a highly positive priming index ($\alpha_p = 0.436$), whereas high-frequency pre-treatment (2.5 kHz) shows a negligible, sub-zero interaction ($\alpha_p = -0.013$). Data values represent cohort means.

Low-Frequency Coupling (250 Hz) yielded a strongly positive priming factor of $\alpha_p = 0.436 \pm 0.022$. This mathematical confirmation indicates that acoustic energy in this band operates synergistically with the cell-membrane potential, accelerating the downstream hydraulic deflation of the pulvinus.

High-Frequency Coupling (2500 Hz) produced a negligible priming metric of $\alpha_p = -0.013 \pm 0.005$. The value centering near zero confirms a total lack of biophysical interaction between high-frequency pressure waves and the stretch-activated ion channel gates.

4. Comprehensive Kinematic Data Matrix

The table below represents the expanded dataset tracking individual replicates ($n = 15$ per cohort) across the precise biophysical markers of the thigmonastic cascade.

Cohort	Replicate ID	Peak Angular Velocity ($\omega_{\max}$, rad/s)	Gating Threshold Energy (E_k , μJ)	Action Potential Latency (L, ms)	Calculated α_p
A (Control)	A-01 to A-05	2.92 ± 0.11	14.2 ± 0.6	185 ± 10	0.000
	A-06 to A-10	2.89 ± 0.14	14.5 ± 0.7	189 ± 12	0.000
	A-11 to A-15	2.95 ± 0.09	13.9 ± 0.5	181 ± 09	0.000
B (250 Hz)	B-01 to B-05	3.74 ± 0.15	8.2 ± 0.4	110 ± 06	0.428
	B-06 to B-10	3.81 ± 0.18	8.5 ± 0.5	115 ± 08	\$0.441\$
	B-11 to B-15	3.79 ± 0.12	8.4 ± 0.3	111 ± 05	0.439
C(2500 Hz)	C-01 to C-05	2.86 ± 0.13	13.8 ± 0.8	178 ± 11	−0.015
	C-06 to C-10	2.90 ± 0.16	14.0 ± 0.7	180 ± 13	−0.007
	C-11 to C-15	2.88 ± 0.11	14.1 ± 0.6	179 ± 10	−0.017

Discussion

1. Biophysical Mechanisms of Frequency-Dependent Gating

The core finding of this study is that pre-exposure to a low-frequency acoustic tone (250 Hz) significantly reduces the mechanical energy required to trigger thigmonastic closure, yielding a highly positive Acoustic Priming Factor ($\alpha_p = 0.436$). Conversely, high-frequency stimulation (2500 Hz) produced an effect statistically indistinguishable from the control baseline. This selective sensitivity suggests that *Mimosa pudica* does not merely register sound as generalized environmental noise; rather, its mechanosensitive infrastructure is finely tuned to a specific acoustic spectrum.

At the cellular level, this phenomenon can be explained by the gating kinetics of Mechanosensitive Ion Channels (MSCs) located within the plasma membranes of the parenchymal cells. Acoustic waves travel as rhythmic mechanical pressure fluctuations. A 250 Hz wave exerts oscillatory shear stress on the cell wall at a frequency that likely matches the resonant properties of the plant's structural proteins or tactile trichomes (plant hairs). This structural resonance causes micro-deformations in the lipid bilayer, inducing a state of sub-threshold mechanical tension.

While this acoustic pressure is insufficient on its own to fully open the channels, it acts as a mechanical "bias." It partially depolarizes the membrane by allowing a minor, localized influx of cytosolic calcium ions (Ca^{2+}). Consequently, when the subsequent tactile stimulus is applied via the pendulum actuator, the energy barrier required to open the voltage-gated channels and initiate a full-scale Action Potential (AP) is significantly lower (8.4 μ J vs. 14.2 μ J in the control). At 2500 Hz, the rapid compression-rarefaction cycles likely occur too quickly for the physical inertia of the lipid bilayer or channel proteins to respond, resulting in no mechanical deformation or priming effect.

2. Kinematic Acceleration and Turgor Efflux Dynamics

The significant increase in peak angular velocity ($\omega_{\max} = 3.78$ rad/s) observed in the 250 Hz cohort indicates that acoustic priming does not just make the plant more sensitive—it accelerates the downstream hydraulic mechanism itself.

Once the tactile threshold is crossed, the velocity of the thigmonastic fold is governed by the rate of water efflux from the abaxial motor cells into the apoplast within the pulvinus. This rapid water movement is driven by an explosive outflux of potassium (K^+) and chloride (Cl^-) ions. Because the acoustic pre-treatment has already stimulated sub-threshold Ca^{2+} signaling, the downstream activation of aquaporins (water channel proteins) and ion pumps occurs with higher synchronicity. Instead of a staggered cellular collapse, the motor cells dump turgor pressure simultaneously, resulting in the accelerated rotational kinematics captured by our high-speed video tractography.

3. Evolutionary Significance and Aneural Cognition

From an evolutionary standpoint, maintaining a state of high tactile sensitivity is metabolically expensive. Rapid leaf closure halts photosynthesis, requires significant ATP to pump ions back into the cells during the recovery phase, and renders the plant temporarily incapable of responding to subsequent threats. Therefore, a permanent state of hyper-sensitivity is an Evolutionary Unstable Strategy (EUS).

Acoustic priming allows *Mimosa pudica* to navigate this trade-off by utilizing predictive environmental cues. In nature, low-frequency vibrations between 100 Hz and 300 Hz are highly salient; they correspond to the wingbeats of heavy insect pollinators, the movement of herbivorous predators, or the approach of heavy rainfall. By "listening" to these low frequencies, the plant can transition into a temporary state of alertness. This capacity to use a non-threatening, predictive stimulus (sound) to optimize a response to a secondary threatening stimulus (touch) provides compelling empirical support for theories of aneural cognition and ecological decision-making in non-neural organisms (Calvo, 2026).

4. Experimental Limitations and Future Outlook

While the acoustic attenuation chamber minimized anthropogenic noise, complete isolation from ultra-low frequency micro-seisms in a dense urban environment like New Delhi remains challenging. Future iterations of this protocol should utilize an anechoic chamber to map a broader, higher-resolution frequency sweep (e.g., testing at 50 Hz increments from 100 Hz to 1000 Hz) to identify the exact peak resonant frequency of the pulvinus. Furthermore, integrating fluorescent intracellular Ca^{2+} imaging would allow researchers to visually confirm the sub-threshold ion gating hypothesized in this paper, turning a macro-behavioral observation into a confirmed molecular mechanism.

Furthermore, because thigmonastic responsiveness is heavily tied to individual cellular turgor dynamics and genetic factors, the absolute values of the Acoustic Priming Factor (α_p) may vary across different plant accessions and developmental stages. Future multi-generational studies are required to map how individual metabolic baselines influence the scale of this acoustic-thigmonastic coupling.

Conclusion

This study successfully demonstrates that *Mimosa pudica* exhibits a frequency-specific acoustic priming mechanism that directly modulates its rapid thigmonastic defense system. By employing a rigorous biophysical framework and standardized mechanical actuators, we confirmed that low-frequency acoustic waves (250 Hz) systematically lower the energy threshold required for leaf closure while accelerating the subsequent rotational kinematics. The absence of any observable response at a higher frequency (2500 Hz) under identical sound pressure levels confirms that this behavior is a targeted physiological reaction rather than a generalized response to acoustic energy.

Ultimately, this capacity to interpret airborne vibrations as predictive indicators of imminent physical contact represents a highly sophisticated form of non-neural information processing. By transitioning into a temporary state of "alertness" only when specific, ecologically relevant frequencies are detected, *M. pudica* successfully balances the high metabolic costs of rapid motor movement with the evolutionary necessity of anti-herbivore defense. This discovery expands our understanding of plant sensory capabilities, demonstrating that plants do not merely react to the present, but computationally prepare for the immediate future.

Data availability

All data generated or analyzed during this study are included in this published article and/or in the online supplement.

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Author Contributions

Author Contributions: Y.J. is the sole investigator of this study and was responsible for all aspects of the research lifecycle. This includes: conceptualization and theoretical design of the acoustic-thigmonastic priming framework; engineering, calibration, and assembly of the custom bioacoustic chamber and physical pendulum actuator; execution of the experimental cohorts and collection of high-speed video data; development of the Python computer vision pipeline and statistical analysis; and the writing, visualization, and final preparation of this manuscript.

Declarations

Conflict of Interest

Competing Interests Statement: The authors declare no competing financial or personal interests that could inappropriately influence, bias, or perceive to influence the conduct, analysis, or presentation of the research described in this manuscript.

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